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# The Journal of Parasitology

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## CONTRIBUTIONS TO THE STUDY OF PARASITIC PROTOZOA. III.

NOTES ON MYXOSPORIDIA FOUND IN SOME FRESH-WATER FISHES OF  
JAPAN, WITH THE DESCRIPTION OF THREE NEW SPECIES  
(WITH FOUR TEXT FIGURES)

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In a former paper (1915) I described from a morphological as well as developmental point of view, a new species (*Myxobolus toyamai* Kudo) from the branchial lamellae of a carp. The present paper is the result of study upon Myxosporidia found since that time. I am now working on the life-histories of the new species described below with the hope of reporting them later.

### 1. *Myxosoma dujardini* Thel.

Eight cysts (the largest being  $200\mu$  in diameter) of round shape, were found in the branchial lamellae of a carp 23 cm. in length. The seat of the parasite was, as in *Myxobolus toyamai*, the connective tissue of the gill-filament.

The results of observations upon the spore coincide for the most part with the description of Thélohan (1895). I wish, however, to give here details about the polar capsule and the polar filament, as Thélohan failed to mention them: length and breadth of the polar capsule  $6-7\mu$  and about  $2\mu$ , respectively, and the length of the polar filament about  $70\mu$ .

### 2. *Zschokkella acheilognathi* n. sp.

Vegetative form. Large ones are generally visible to the naked eye as small, opaque, more or less regular, usually subspherical masses, occupying various parts of the gallbladder and especially of the gall-duct (Fig. 1). The size varies with age up to a maximum length of  $720\mu$  by a breadth of  $550\mu$ , and the thickness of one individual is about uniform throughout, but in many specimens it differs from 5 to  $30\mu$  according to the size of the myxosporidium. Their bodies are very flexible and easily doubled up, representing, in sections, various forms. The vegetative stage in sections resembles much that of *Sphaeromyxa*

*hellandi*, observed by Auerbach (1912), both in form and structure. The body is colorless in both young and old. In its fresh condition the protoplasm can be seen to be clearly differentiated into finely granulated reticular ectoplasm and greatly vacuolated endoplasm. In the younger form (15 to 30 $\mu$  in greatest diameter) lobose pseudopodia are well developed. The myxosporidium moves about more or less actively in the bile by the constant emission of pseudopodia. No clear evidence of the active emission of pseudopodia exists in older individuals. The ectoplasm of some more advanced specimens shows in section two structures; the outer layer, comparatively thin but uniformly about 2 $\mu$  in thickness, presents very fine striations, while the remaining part is finely alveolated, having an average thickness of 6 to 8 $\mu$ .

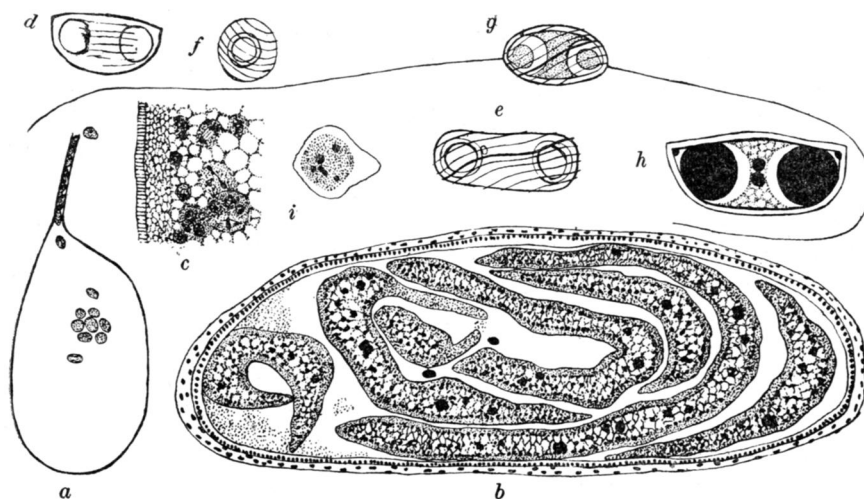


Fig. 1.—*Zschokkella acheilognathi* n. sp. *a*, Gall-bladder of *Acheilognathus* with many myxosporidia,  $\times 10$ ; *b*, oblique cross section of the infected gall-duct,  $\times 200$ ; *c*, part of the cross section of the parasite, showing the differentiation of the protoplasm,  $\times 1,000$ ; *d*, to *g*, spores: in *g*, filaments are extruded,  $\times 1,500$ ; *h*, stained spore, about  $\times 2,250$ ; *k*, young myxosporidium,  $\times 1,000$ .

Auerbach (1910b) seems to have observed similar structure in the outer layer of the ectoplasm in *Sphaeromyxa hellandi*, sketching a surface view of an individual fixed in formol. It takes stains much more deeply than the endoplasm, so that in sections it shows clear differentiation of the protoplasm much better than was shown in *Myxobolus toyamai*. The endoplasm contains vegetative nuclei as well as generative nuclei in several stages of spore formation. It is polysporous, according to the observation made up to the present time. In this regard, it is quite different from *Zschokkella hildae*, in which, after Auerbach (1910a), single and double spore formation occurs.

Spore. Generally oval with round poles, very often more or less

hemispherical, somewhat attenuated symmetrically at both ends of the flat side of the spore. Several modifications in form and size, however, are also found in the present case as in other forms. It is usually 10 to 14 $\mu$  long by 6 to 7 $\mu$  wide. The thickness is almost equal to the width. Shell bivalve; the line of junction being oblique to the longitudinal axis of the spore. Parallel to the line of junction, fine striations run longitudinally on the spore coat. A polar capsule, round in shape, with a diameter of 2 to 3 $\mu$ , at each end of the spore, takes stains very deeply. Polar filaments were easily extruded by the application either of mechanical pressure or KOH-solution, and are well stained after my method (1913). The fully extruded polar filaments were 65 to 70 $\mu$  long.

Habitat. This species is found quite abundantly in the gallbladder of *Acheilognathus lanceolatum* Temm. et Schl., commonly found in brooks in the vicinity of Tokio. Out of twenty-four fish (8 to 12 cm. long) examined in May, 1915, twenty-one were found to have harbored the parasite; thus the rate of the infection rises above 80 per cent. Matured, large vegetative forms were very often found in great numbers in the gallduct, while in the gallbladder of the same host I could find only a small number of isolated spores. Klokacewa (1914) described a somewhat similar form of spore from *Carassius vulgaris*, without finding the vegetative form. The species in question, though its vegetative form differs apparently from that of *Zschokkella hildae* seems to belong to that genus. Up to the present time two species of the genus have been reported, that is, *Zsch. hildae* and *Zsch. nova*, since Auerbach (1910) created it. The former apparently differs from the present form in several points. Now the dimensions of the spore of the latter seem to correspond very nearly to the myxosporidium in question. As it lacks, however, all other details, it is impossible to make accurate identification, so I treat this species as a new one, calling it *Zschokkella acheilognathi*.

### 3. *Myxobolus fuhrmanni* Auer

Isolated spores of this form occur very often in the bile of the loach (*Misgurnus anguillicaudatus*). The vegetative form has not yet been found by me. The description of the spore by Auerbach (1909) coincides well with my observations, except that I found the thickness of the spore coat to be uniform, whereas Auerbach's observation was in effect that the shell is especially thick at the posterior end of the spore. I wish to mention that the length of the polar filament in the present case is 100 $\mu$ . Nearly 50 per cent. of the said fish, examined in September, 1915, were infected by this *Myxobolus*. In all cases, however, the infection seems to be carried to a very slight degree.

4. *Myxidium* sp.

This form, together with the following two new species, are also found in the gallbladder of the loach. The vegetative form has not yet been observed. Two per cent. of the fish studied in September, 1915, were infected. The spores are mostly found separated from each other, floating in the bile. One side of the spore is, in most cases, more convex than the other. The sporoplasm usually occupies the whole inner space of the spore, except the polar capsules, and shows fine granulations in the natural condition as in the case of *Myxidium giardi* or *Myxobolus pfeifferi*, and also shows a fine alveolar structure in stained preparations. It contains two nuclei of almost equal size. Shell bivalve, the line of junction of which is straight. On the surface of the shell, fine striations run longitudinally parallel to the line of junction. The dimensions are: length, 15 to 18 $\mu$ , breadth 6 to 7 $\mu$ , length of polar capsule 7 to 8 $\mu$ , and that of polar filament 60 to 70 $\mu$ .

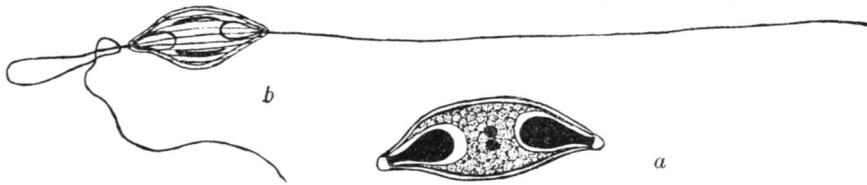


Fig. 2.—*Myxidium* sp. *a*, stained spore,  $\times 1,750$ ; *b*, spore with extruded polar filaments,  $\times 1,000$ .

Ishii (1915) recently described a new species, *Myxidium anguillae*, from an eel. The form in question apparently differs from any of the species reported up to the present time. I hope to identify the species after studying it more closely.

5. *Chloromyxum misgurni* n. sp.

Vegetative form. Mostly round, often of irregular form. From a side view it assumes a semicircular shape. From the more or less flat surface many fine root-like pseudopodia extend. They are more clearly visible in younger specimens, where the spore formation has not yet begun. There is no clear differentiation of protoplasm. It is finely vacuolated on the whole. With the pseudopodia, the myxosporidium probably creeps along the surface of the epithelial layer of the gallbladder, so that many individuals in different stages of development are found in section preparations, closely attached to the epithelial cells with their peculiar pseudopodia. The size varies with age, the largest being 50 $\mu$  in greatest diameter, with the maximum thickness of 20 $\mu$ . Individuals with six to eight spores are of common occurrence; those with twelve to sixteen spores, however, occur rarely, and it is very seldom that only two spores are found in one myxosporidium.

Spore. Spherical, slightly attenuated at the anterior end. Shell

bivalve; the line of junction straight. Parallel to the ridge which marks the line of junction very clearly, run fine longitudinal striations. Four polar capsules are situated in the anterior end. In the finely granulated sporoplasm two nuclei of equal size are found. The dimensions of the spore are: length, 8 to  $9\mu$ , breadth 6 to  $7\mu$ , thickness 5 to  $6\mu$ , length of the polar capsule 2 to  $3\mu$  and that of polar filament 28 to  $35\mu$ .

Habitat. In the gallbladder of *Misgurnus anguillicaudatus* Cantor.

Of the fish examined in September, 1915, 73 per cent. were found to be infected.

Of forms known up to the present time, *Ch. fluviatile* (Thélohan, 1895) seems to be nearest in size and form of the spore and seat of infection to the *Chloromyxum* mentioned. The form and size of the

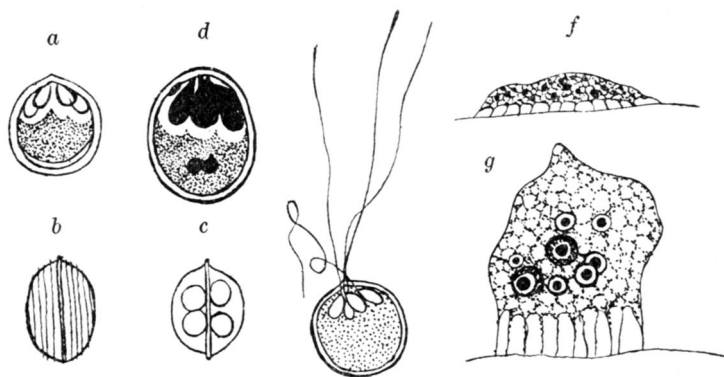


Fig. 3.—*Chloromyxum misgurni* n. sp. a, front view; b-c, side view of the spore in natural condition,  $\times 1,750$ ; d, stained spore,  $\times 2,625$ ; e, spore with the extruded polar filaments,  $\times 1,750$ ; f, g, two young myxosporidia in section,  $\times 1,750$ .

pseudopodia and the structure of the spore of the type studied differ from that described by Thélohan. Therefore, I propose to name this *Chloromyxum misgurni*.

#### 6. *Chloromyxum fujitai* n. sp.

Vegetative form. Mostly round, sometimes irregular. There is no clear differentiation of the protoplasm. The endoplasm is highly vacuolated, the ectoplasm being hardly visible. The largest one was  $40\mu$  in diameter. They float about in the bile, so that in sections of infected gallbladder they are found in the gall apart from the epithelial layer, by which fact we can easily distinguish them from *Chloromyxum anguillicaudati*, even when both forms occur in the same gallbladder. Disporous and polysporous, with up to eight spores.

Spore. Circular in general; often attenuated at the anterior end. Shell bivalve; the line of junction not being straight, but very thick.

The shell has peculiar thick ridges running longitudinally on the surface. Near the anterior end of the spore two small circular markings are clearly visible, in preparations well stained with Heidenhain's iron hematoxylin, one on either side of the line of junction, from which the markings recede on both valves. These two circular markings are not the exits for polar filaments, because it is clearly shown in preparations stained with Giemsa's solution that the four polar capsules have their independent exits. The form of the spore takes different aspects by the presence of the characteristic markings resembling partially those of *Hoferia cyprini* (Doflein, 1898) and *Chloromyxum koi* (Fujita, 1913). In optical cross-section, the spore represents an outline quite like a cog-wheel with twenty to twenty-two ridges, including the widest ridges, which mark the line of junction of the shell. The

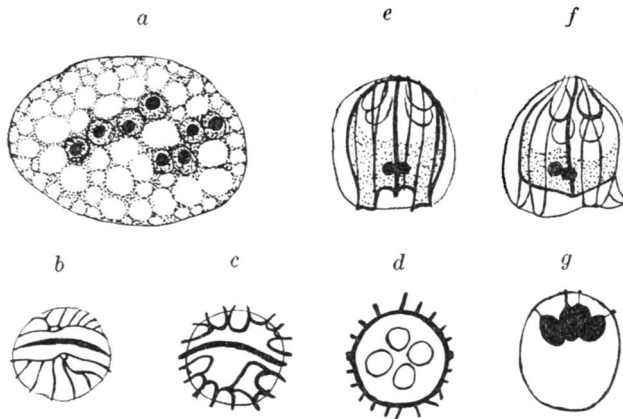


Fig. 4.—*Chloromyxum fujitai* n. sp. *a*, young vegetative form,  $\times 1,750$ ; *b*, *c*, anterior (*b*) and posterior (*c*) view of a stained spore,  $\times 1,750$ ; *d*, optical cross section of the same spore as the above,  $\times 1,750$ ; *e*, *f*, side views of stained spores,  $\times 1,750$ ; *g*, side view of stained (Giemsa) spore,  $\times 1,750$ .

thickness of the ridges varies regularly. The thickest ones are located where a plane perpendicular to that of junction cuts the shell longitudinally, others decreasing in thickness as they approach the line of junction. Four polar capsules occupy the anterior half of the spore. The sporoplasm contains two nuclei of almost equal size. The dimensions are: length 10 to 12 $\mu$ , breadth 8 to 10 $\mu$ , length of polar capsule 2 to 3 $\mu$ , and that of the polar filament 23 to 30 $\mu$ .

**Habitat.** In the contents of the gallbladder of *Misgurnus anguillicaudatus*. The occurrence is much rarer than that of the former one, showing about 5 per cent. in September, 1915.

Of all descriptions from this genus that of Fujita (1913) alone described similar markings of the spore of *Chloromyxum koi* from the gallbladder of the carp. However, we find a great difference

in form and in the number of ridges. There is also a great difference between the size and structure of the spore, and in the number of the spores found in a vegetative form. So I think this species is a new one. In honor of Dr. T. Fujita, who was the first to study myxosporidia in Japan and to discover the spore of this type, I give the name *Chloromyxum fujitai*.

Multiple infection of the above-mentioned four species takes place very often.

Concerning the pathological effects, I have but little to report. No visible external change could be noticed in any of the infected fishes. But, as in the case of *Acheilognathus*, we found often that the gall-duct had been filled up with a great number of the *Zschokkella* (Fig. 1), therefore it is certain that the secretion of the bile into the duodenum must be greatly disturbed. Such is the case with the highly infected loach, the gallbladder of which has an opaque appearance. It is, however, difficult at present to state the real effects of the parasite upon the host.

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